USE OF GENOMICS IN THE IMPROVEMENT OF CROPS

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PROBLEMS FACING THE 21ST CENTURY

- New diseases/pathogens
- Old diseases
- Climate change/global warming
- Astronomic population growth
- Dwindling world resources
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agriculture

- Increase yields
- Low/er chemical inputs
- Stress resistance
  - Abiotic (drought, heat, etc)
  - Biotic (disease, pest, etc)
- Sustainability
- Improved value-added
agriculture

genomics
Genomics: the study of the genome which is the sum total of all heritable sequences in an organism, DNA in most cases, and all its attributes.
Traits that are important in agriculture are dictated by genes annotated from DNA sequences.
Increase yields
Low/er chemical inputs
Abiotic stress resistance
Biotic stress resistance
Sustainability

agriculture

genomics

Gene sequence information
Current research in Agriculture

Improvement of crop production through
• traditional breeding
Current research in Agriculture

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- traditional breeding ➔ SLOW
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- marker-assisted selection
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Improvement of crop production through

• traditional breeding
• marker-assisted selection
• transgenic technology
• RNAi technology
• optimization of chemical inputs and soil conditions
Current research in Agriculture

Improvement of crop production through

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- marker-assisted selection ➔ BETTER BUT DOES NOT WORK ALL THE TIME
- transgenic technology ➔ LIMITED KNOWLEDGE ON GENE SEQUENCES
- RNAi technology ➔ LIMITED KNOWLEDGE ON GENE SEQUENCES
- optimization of chemical inputs and soil conditions (crops) ➔ RELIES HEAVILY ON FERTILIZERS, PESTICIDES, ETC
Traditional breeding
• Selects for desired trait/s by crossing and waiting for the progeny to grow and manifest phenotype
Traditional breeding for high yield and disease resistance

- Disease resistant, but low yielding
- Disease susceptible, but high yielding

Several generations, i.e., years of crosses
Marker-assisted selection

- Uses molecular markers to breed for desirable traits
- Markers may be SSRs, RFLPs, AFLPs, etc
- Usually applies to quantitative trait loci or quantitative traits (QTLs)
QTLs
• Traits whose expression are not determined by a single gene or locus
• Mapping and breeding these traits are a big challenge
• LOD - log of odds
  - log of the probability that the evidence can explain a phenomena/the probability that it is a random event
Examples of LOD

a) 1/1
   LOD = 0

b) 1/0.1
   LOD = 1

c) 1/0.01
   LOD = 2

A cut-off of LOD 3 is usually accepted as support for linkage in genetics.
Examples of quantitative traits

- maize resistance to *Aspergillus flavus*
Examples of quantitative traits

- maize resistance to *Aspergillus flavus*
- rice resistance to bacterial panicle blight
Transgenic technology

• aka genetic engineering technology
• transfer of a gene or a part thereof into the same or another organism

• but we need to know the gene sequence
RNA interference

- aka gene silencing
- a mechanism acquired by genomes to ward off virus infection and transposons
RNA interference

- aka gene silencing
- a mechanism acquired by genomes to ward off virus infection and transposons
- again, we need gene sequence
Optimization of chemical inputs and soil conditions

- focused on effects of inputs on yield and not on physiological properties of crops
- if crop physiology is optimum for nutrient absorption, minimum input would be needed
- knowledge of gene sequence for a specific physiological property would be extremely helpful
Optimization of chemical inputs and soil conditions
Current research in Genomics

- Genome sequences of different
  - animals including livestocks
  - plants including crops
  - plant and animal pests
  - plant and animal pathogens
- Transcriptomic sequences of different processes associated with above organisms
- Proteomic sequences of different processes associated with above organisms
- Metabolomic sequences associated with above
- Transcriptomics - study of the transcriptome which is the sum total of mRNA expression from a specific time point in the life cycle of an organism under a specific condition
- Proteomics - study of the proteome which is the sum total of protein expression from a specific time point in the life cycle of an organism under a specific condition
- Metabolomics - study of the metabolome which is the sum total of all metabolomes secreted from specific time point in the life cycle of an organism under a specific condition
Crops with completed or partially completed genomes

- Rice
- Cottonwood
- Grapes
- Sorghum
- Maize
- Soybean
- Apple
- Strawberry
- Cacao
- Date palm
- Potato
- Chinese cabbage
- Alfalfa
- Pigeonpea
- Cassava
- Foxtail millet
- Tomato
- Melon
- Banana
- Orange
- Cotton
- Bamboo
Crops with completed or partially completed genomes

- Earlier projects used BAC libraries and dideoxy sequencing
- Later ones used NGS and whole genome shotgun techniques
- High-throughput data analysis methods were used for annotation
- Data sequences and annotations were submitted to public databases for public use
Data sequences and annotations may be used as precise molecular markers via PCR or qPCR.
Data sequences and annotations may be used as precise molecular markers via PCR and sequencing.

SNP1 (desirable trait)  

```
CCCGTTAGGTAACCTTGGAAAGCGA
```

SNP2 (undesirable trait)  

```
CCCGTTAGATAACCTTGGAAAGCGA
```
Data sequences and annotations may be used as target/s to focus on gene function studies (aka as reverse genetics)

- Known gene sequences may be used as markers, transgenes or RNAi sequences
Characteristics of crop genomes

• Mostly huge
  • Number of genes almost the same
  • Repetitive elements content makes up the difference
• Notorious for polyploidy
• However, gene order or synteny occurs
Synteny
Application of synteny
Sequencing of crop genomes

- Greatest challenge is genome size
- Exome sequencing an alternative
- Use synteny to study big genomes that are not accessible via WGS
- Use resources such as BACs to search for genes of interest
Gene search in loblolly pine using BAC resources

Probes used

cell wall synthesis
  cellulose synthase
  cinnamyl alcohol dehydrogenase
  expansin (alpha)
  fasciclin-like AGP
  glucomannan synthase
  glycoside hydrolase family 28
  Korrigan endoglucanase
  laccase
  lignin related
  phenylalanine ammonia lyase
  sucrose synthase
  UDP glucose pyrophosphorylase
  UGP mannose pyrophosphorylase
  vein patterning

transcription factors
  assymetric leaves-like; LOB domain transcription factor
  brassinosteroid responsive transcription factor
  brassinosteroid-insensitive; shaggy-like protein kinase
  bZIP transcription factor
  HD Zip III
  Kanadi, Myb-related protein
  KNOX homeobox transcription factor
  LIM domain protein
  Myb family transcription factor
  NAC domain
  R2R3-MYB transcription factor
  transcription factor WRKY1

disease resistance
Gene search in loblolly pine using BAC resources

Masked repeats using Repeatmasker

Retrotransposons the most abundant repeats

BLAST, FGenesh, Augustus and GeneMark to predict genes and Artemis to view predictions
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Annotation of 114 gene models

9 predicted genes

105 pseudogenes
Benefit from pathogen and pest genome sequences

- Provide clues on pathogenicity, virulence and/or infectivity
  - May assist breeding programs on what to select
- May afford gene sequences for use in transgenic and RNAi techniques
Benefit from transcriptomic/proteomic/metabolomic research

- Allow for a better understanding of the biology of an organism
- May assist breeding programs on what to select under a specific condition
- May afford additional information on gene and protein expression
- Aid in identifying mechanisms associated with a process for the organism
Bacterial panicle blight
Bacterial panicle blight (BPB)

- Caused by *Burkholderia glumae*
- First observed in Asia in the 50’s and in the late 90’s in the U.S.
- Aggravated by hot and humid conditions
- May cause up to 70% in yield loss
- No reliable source of resistance known
- Disease cycle and pathogenicity unknown
Searching for mechanism of resistance

CL161
Resistant rice seedlings and

CL151
Susceptible rice seedlings

Water inoculated

B. glumae inoculated

sample after 48 hours

Total RNA

Total RNA

Total RNA

Total RNA

host transcriptome

host transcriptome

host transcriptome

host transcriptome

pathogen transcriptome

pathogen transcriptome

(pathogen transcriptome (3 reps)

miRNAome

miRNAome

miRNAome

miRNAome (2 reps)
A cursory look at the DE transcripts

- Known resistance genes (transcripts) against rice blast and bacterial blight are not expressed at the inoculated condition.
- Different kinds of defense response transcripts are up-regulated in the resistant and susceptible genotypes.
PIF-like ORF1

- P instability factor
- Class 2 transposable element
- Constitutively expressed in resistant line
- PIF has 2 ORFs
  - ORF2 is transposase (not found)
  - ORF1 probably involved in DNA and protein binding
NBS-LRR resistance genes

- Ancient
- Involved in pathogen recognition
- Present in large numbers
  - Occurs in clusters (most cases)
  - A lot are pseudogenes (illegitimate recombination)
- Usually negatively regulated
  - Activated by ATP
Gene Ontology annotation of differentially expressed transcripts
Quantitative RT-PCR Validation
Conclusion (transcriptomic section):

1. Known resistance genes against rice blast and bacterial blight are not expressed in the resistant genotype; some not even in the water control.

2. Transcripts involved in pathogen response are constitutively expressed in resistant genotype. They include activation partners and other downstream components of the resistance pathway.

3. A once part of a transposable element appeared to have been co-opted as a defense arsenal.

4. Resistance may have existed prior to rice domestication.
Public Databases

- cell phones, tablets, etc

Details on how to grow, care and select for a genotype of a crop on a specific environment under a specific set of conditions.
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